REMARKS

Status of the Claims

Claims 1, 12, 21-23, 31-34, 37-42, 44-48, 50-55, 58-65 and 67-72 were currently pending. Claims 1, 23, 31, 39, 45, 50, 60, and 68 have been amended to recite "a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2." Support for the amendments to the claims can be found, *inter alia*, at paragraphs **[0018]**, **[0040]**, and **[0041]**. Claims 45 and 68 were also amended to delete duplicative words for clarity. No new matter is added by the amendments. The remaining claims have not been further amended. After entry of the amendment, claims 1, 12, 21-23, 31-34, 37-42, 44-48, 50-55, 58-65 and 67-72 will be pending.

This application pertains to compositions and assays for sodium and lithium ions using ion-sensitive enzymes. In particular, the ion-sensitive enzyme is a chimeric protein comprising a specific bacterial leader sequence, a 3'(2'), 5"-bisphosphate nucleotidase of SEQ ID NO:2 (or having one conservative amino acid substitution and at least 90% of the bisphosphonate activity of SEQ ID NO:2); and a fragment comprising the sequence of SEQ ID NO:3. The bisphosphonate activity is the activity of forming inorganic phosphate and AMP from adenosine 5' biphosphate. (See paragraph **[0043]** of the specification).

The outstanding issues are written description and enablement – specifically, whether the claim limitation "a second peptidyl fragment comprising the amino acid sequence . . . of SEQ ID NO:2 having one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2" meets the written description and enablement requirements.

The claims were not rejected over the prior art.

Entry of the amendment and reconsideration in view of the following comments is respectfully requested. With respect to all amendments, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Interview Summary

The undersigned appreciates the courtesy extended by the Examiner in the telephonic interview. Applicants hereby acknowledge the summary of the interview conducted with the Examiner, and agree with the substance of the interview as described by the Examiner.

Claim 23

Claim 23 was not rejected or objected to in the previous (12.16.2009) Office Action, and accordingly, Applicants submit that this claim should have been indicated as allowable.

Rejection Under 35 U.S.C. §112, first paragraph, written description

Claims 1, 12, 21, 22, 31-34, 37-42, 44-48, 50-55, 58-65, 67-72 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Office has taken the position that the claims do not place any limitation upon the number of conservative amino acid substitutions that may be made to SEQ ID NO:2. (12.16.2009 OA at page 5.) Accordingly, the Office asserts that the claimed chimeric proteins comprise an enormous number of species.

The written description requirement "may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure" and compliance with the requirement "is essentially a fact-based inquiry that will 'necessarily vary depending on the nature of the invention claimed.'" See *Amgen, Inc. v. Hoechst Marion Roussel, Inc. and Transkaryotic Therapies, Inc.*, USPQ 65 USPQ2d 1385 (Fed. Cir. 2003); *Enzo Biochem, Inc. v Gen-Probe, Inc.*, 296 F.3d 1316, 63 USPQ2d 1609 (Fed. Cir. 2002).

Applicants respectfully traverse this rejection in light of the amendments to the pending claims, and in view of the revised guidelines concerning compliance with the written description requirement.

Claim 1 is directed to an isolated chimeric protein having the enzymatic activity of a nucleotidase, which chimeric protein comprises, from N-terminus to C-terminus, a first peptidyl fragment comprising a bacterial leader sequence comprising an amino acid sequence as set forth in SEQ ID NO:1; a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2.; and a third peptidyl fragment comprising an amino acid sequence as set forth in SEQ ID NO:3. The other pending claims all contains this same claim language.

Applicants assert that conservative amino acid substitutions of amino acid sequences were well-known in the art, and were fully described in the specification at paragraph **[0018]**. A person of ordinary skill in the art could empirically replace one amino acid in SEQ ID NO:2 with a conservative amino acid substitution, and test the resultant peptide to determine whether it has retained the requisite 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2. Thus, the pending claims place a numerical limitations upon the number of conservative amino acid substitutions that may be made to SEQ ID NO:2.

Moreover, SEQ ID NO:2 is 356 amino acids in length. Changing one amino acid out of this 356 amino acid sequence would result in a peptide having 99.72%[1] sequence identity to SEQ ID NO:2. As stated in Example 10 of the revised written description guidelines, "In view of the disclosure of SEQ ID NO: 3, those skilled in the art could readily envision all of the amino acid sequences that are 95% identical to SEQ ID NO: 3. Those skilled in the art could recognize amino acid sequences that are 95% identical to SEQ ID NO: 3 by comparing a given sequence to SEQ ID NO: 3. The presence of an amino acid sequence that is at least 95% identical to SEQ ID NO: 3 is a structural feature of each of the proteins within the claimed genus." The same applies here: those skilled in the art could readily envision all the amino acid sequences that are 99.72% identical to SEQ ID NO:2, and could recognize sequences based upon this structural similarity. A person of skill in the art could routinely make and identify variants having 99.73% identity to SEQ ID NO:2.

A person of skill in the art, reading the specification, would be able to design such peptides having 99.72% sequence identity to SEQ ID NO:2, and test the derivative peptides for the requisite activity.

This type of testing is described in the specification, for example at paragraph **[0043]**, which states:

> Assays for enzymatic activities of 3'(2'),5'-bisphosphate nucleotidases are known in the art (*See e.g.*, Murguía et al., *J. Biol. Chem.*, 271(46):29029-33 (1996)). Exemplary methods for phosphatase activity include determining the formation of inorganic phosphate (Pi) and AMP include colorimetric methods (*See, e.g.*, Gumber et al., *Plant Physiol.*, 76:40-44 (1984); Baykov et al., *Anal. Biochem.* 171:266-70 (1988)) and radioactive-labeled substrates (*See, e.g.*, Spiegelberg et al., *J. Biol. Chem.* 274(19):13619-28 (1999); Peng et al., *J. Biol. Chem.* 270(49):29105-29110 (1995)).

[1] (355 ÷ 356) x 100 = 99.72%

A person of skill in the art, reading the specification, would be able to design and test the derivative peptides for the requisite 90% activity, particularly since there is an art recognized structure function relationship.

The art discloses sufficient relevant identifying characteristics such that a person of skill would be able to correlate the structure of SEQ ID NO:2 with the requisite functional activity as described in detail in Example 11B of the written description guidelines. Example 11B of the revised written description guidelines pertains to the situation where the specification (or art) while not disclosing any nucleic acid sequences that encode a polypeptide with novel activity Y other than the claimed sequence, does identify domains critical for activity, and proposes that conservative amino acids will result in protein having the claimed activity. The written description guidelines state that :

> [a]lthough all conservative amino acid substitutions in these domains will not necessarily result in a protein having activity Y, those of ordinary skill in the art would expect that many of these conservative substitutions would result in a protein having the required activity. Further, amino acid substitutions outside of the two identified functional domains are unlikely to greatly affect activity Y. Thus, a correlation exists between the function of the claimed protein and the structure of the disclosed binding and catalytic domains. Consequently, there is information about which nucleic acids can vary from SEQ ID NO: 1 in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. Based on the applicant's disclosure and the knowledge within the art, those of ordinary skill in the art would conclude that the applicant would have been in possession of the claimed genus of nucleic acids based on the disclosure of the single species of SEQ ID NO: 1.

This same type of information in the art is available for SEQ ID NO:2, such that there is information about which amino acids can vary from SEQ ID NO:2 while retaining the requisite activity.

SEQ ID NO:2 is the Hal2p protein. This protein was known in the art at the time the application was filed, as evidenced by paragraph **[0040]**. Furthermore, the art recognized the functional domains of Hal2p proteins. For example, Albert et al.(*X-ray Structure of Yeast Hal2p, a Major Target of Lithium and Sodium toxicity, and Identification of Framework Interactions*

Determining Cation Sensitivity, J. Mol. Biol. (2000) 295:927-938, **Exhibit A**) solved the crystal structure of Hal2p complexed with magnesium, lithium AMP and Pi. Hal2p is a two-domain structure linked at residue 220, containing an N-terminal domain, and a C-terminal domain (*id.,* at page 928, right column). The active site lies between the N-terminal and C-terminal domains, and is capped by a hairpin that includes residues 34-45 (*id.*, page 928 – 929). Albert noted that the crystal structure of members of the Hal2p superfamily has a similar structure, where the metal sties are structurally conserved at the intersection of topologically equivalent secondary structures (*id.,* page 929). Albert found that magnesium appears to occupy sites S1 and S3, lithium occupies site 2; and AMP occupies two pockets in the C-terminal domain (*id.,* page 929). Furthermore, Albert describes residues identified as affecting lithium sensitivity, which correspond to Lys33, Glu72, Asp142, Asp145, and Thr147 in Hal2p (*id.,* page 929, citing to Pollack et al., 1993, Gore et al., 1993, Rees-Milton et al., 1997). So, for example, mutations outside of those residues identified as being important for lithium sensitivity are unlikely to affect the lithium binding activity of the protein. A skilled artisan at the time of the invention would have understood that high conservation of amino acid sequences typically has important functional implications, and therefore highly conserved amino acids within the family should not be mutated if one desires to retain biological function, or alternatively, biological function is more likely to be retained if conservative substitutions are made in these regions. Clearly, a large of amount of structure-function correlation data had been published at the time the application had been filed.

Based on the foregoing discussion of this rather limited selection of prior art, a person skilled in the art would have inferred that, being a member of the family of phosphatases that hydrolyze PAP to AMP, the Hal2p protein is subject to certain well-established rules with respect to catalytic activity. One of skill in the art upon reviewing Albert (and the other references cited in the specification), would have been provided with adequate guidance with respect to making functional mutants of Hal2p containing one conservative amino acid substitution, and that retain a high (90%) level of 3'(2'),5'-bisphosphonate activity.

In light of the foregoing discussion, Applicants respectfully submit that the specification, combined with the knowledge in the art at the time of the present invention, provides sufficient

disclosure to convey to a person skilled in the art that Applicants were in possession of the claimed invention. Accordingly, Applicants respectfully submit that this written description rejection under 35 U.S.C. § 112, first paragraph may properly be withdrawn.

Rejection Under 35 U.S.C. §112, first paragraph, enablement

Claims 1, 12, 21, 22, 31-34, 37-42, 44-48, 50-55, 58-65 and 67-72 stand rejected under 35 U.S.C. §112, first paragraph as allegedly failing to meet the enablement requirement.

The Office has taken the position that because any unlimited number of amino acid substitutions may be made in the claimed peptide, that the specification does not provide the requisite level of guidance. (12.16.2009 OA at page 9.) In addition, the Office stated that Applicants previous arguments are not persuasive since "the specification does not establish: (A) regions of the protein structure which may be modified without 3'(2'),5'- bisphosphonate activity of SEQ ID NO:2; (B) the general tolerance of SEQ ID NO:2 to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any 3'(2'),5'-bisphosphonate residue with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful." (12.16.2009 OA at page 10.)

The law on enablement has been set out in responses to previous office actions, which are incorporated hereby in their entirely.

Applicants traverse this rejection. Compliance with 35 U.S.C. § 112, first paragraph enablement does not require that specific portions of any amino acid sequence be identified. However, it does not require undue experimentation to determine those portions of the sequence that are capable of mediating a biological function similar to that mediated by the protein of SEQ ID NO:2. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *Telectronics* at 785 (emphasis added). Nothing more than objective

enablement is required, and it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Some experimentation is allowed as long as it is not undue.

Claim 1 is directed to an isolated chimeric protein having the enzymatic activity of a nucleotidase, which chimeric protein comprises, from N-terminus to C-terminus, a first peptidyl fragment comprising a bacterial leader sequence comprising an amino acid sequence as set forth in SEQ ID NO:1; a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2.; and a third peptidyl fragment comprising an amino acid sequence as set forth in SEQ ID NO:3. The other pending claims all contains this same claim language.

Accordingly, the pending claims encompass chimeric proteins comprising SEQ ID NO:4, and chimeric proteins having a substituted peptidyl fragment having one conservative amino acid substitution of SEQ ID NO:2 (contained in SEQ ID NO:4) where substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2. The pending claims do not recite an unlimited number of amino acid substitutions. A person of ordinary skill in the art could empirically replace one amino acid in SEQ ID NO:2 with a conservative amino acid substitution, and test the resultant peptide to determine whether it has retained the requisite 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2. Thus, the pending claims place a numerical limitations upon the number of conservative amino acid substitutions that may be made to SEQ ID NO:2. It would not take undue experimentation to arrive at this number of SEQ ID NO:2 mutants.

The art of preparing a polypeptide with a conservative amino acid mutation compared to another polypeptide having a fully defined sequence and a certain type of known biological activity was well-settled and routine at the time the present application was filed. The specification expressly describes methods by which such polypeptides having conservative amino acid mutations can be prepared without any undue experimentation. For example, the specification teaches the types of amino acid substitutions that may be used to achieve functional equivalence (paragraph

[0018]). Numerous computer programs exist that simplify the task of designing homologous nucleotide sequences that are likely to have similar biological activities through conservative amino acid substitutions. The actual preparation of the nucleic acids that encode such conservative variants also involves routine automated steps.

SEQ ID NO:2 is 356 amino acids in length. Substituting one amino acid out of this 356 amino acid sequence would result in a peptide having 99.72%[2] sequence identity to SEQ ID NO:2.

As described previously, in the recent *Kubin* appeal stemming from U.S. Appl. No. 09/667,859, the exemplary claim recited "[a]n isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide at least 80% identical to amino acids 22-221 of SEQ ID NO:2, wherein the polypeptide binds CD48." The Examiner rejected the claim for lack of enablement. The Board of Patent Appeals and Interferences overturned the enablement rejection while concluding that "[t]he amount of experimentation to practice the full scope of the claimed invention might have been extensive, but it would have been routine [because] [t]he techniques to do so were well known to those skilled in the art." *Ex parte Kubin*, Appeal No. 2007-0819, at 14 (BPAI May 31, 2007).

Applicants' claims are directed to chimeric proteins having one conservative amino acid substitution of SEQ ID NO:2, which is far fewer than the roughly 40 amino acid changes found enabled by the Federal Circuit in *Kubin*. (12.16.09 OA at page 12). Applicants' claims are thus narrower in this respect than *Kubin's* claims.

A person of skill in the art, reading the specification, would be able to design such peptides having 99.72% sequence identity to SEQ ID NO:2, and test the resultant peptides for the requisite activity. Paragraph **[0043]** of the specification discloses specific assay protocols that can be used to evaluate the biological activity of mutant recombinant proteins. Testing peptides with once conservative substitution compared to SEQ ID NO:2 using the disclosed assays and comparing them to the reference proteins is routine and does not require undue experimentation.

---

[2] (355 ÷ 356) x 100 = 99.72%

The Office is concerned that the specification contains insufficient teachings relating to "(A) regions of the protein structure which may be modified without 3'(2'),5'- bisphosphonate activity of SEQ ID NO:2; (B) the general tolerance of SEQ ID NO:2 to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any 3'(2'),5'-bisphosphonate residue with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful." (12.16.2009 OA at page 10.)

The specification need not disclose what is well-known in the art. (*See* MPEP §2164.08, stating "not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted." *Citing to In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991).)

Here, the art satisfies those teachings identified by the Examiner. A person of skill in the art, reading the specification, and with knowledge of the art, would be able to design and test the derivative peptides for the requisite 90% activity, since there is an art-recognized structure function relationship. The art contains information about which amino acids can vary from SEQ ID NO:2 while the peptide retains the requisite activity.

SEQ ID NO:2 is the Hal2p protein. This protein was known in the art at the time the application was filed, as evidenced by paragraph **[0040]**.

The functional domains of Hal2p proteins were also known. For example, Albert et al.(*X-ray Structure of Yeast Hal2p, a Major Target of Lithium and Sodium toxicity, and Identification of Framework Interactions Determining Cation Sensitivity*, J. Mol. Biol. (2000) 295:927-938, **Exhibit A**) solved the crystal structure of Hal2p complexed with magnesium, lithium AMP and Pi. Hal2p is a two-domain structure linked at residue 220, containing an N-terminal domain, and a C-terminal domain (*id.,* at page 928, right column). The active site lies between the N-terminal and C-terminal domains, and is capped by a hairpin that includes residues 34-45 (*id.*, page 928 – 929). Albert noted that the crystal structure of members of the Hal2p superfamily has a

similar structure, where the metal sties are structurally conserved at the intersection of topologically equivalent secondary structures (*id.,* page 929). Albert found that magnesium appears to occupy sites S1 and S3, lithium occupies site 2; and AMP occupies two pockets in the C-terminal domain (*id.,* page 929).

Furthermore, Albert describes residues identified as affecting lithium sensitivity, which correspond to Lys33, Glu72, Asp142, Asp145, and Thr147 in Hal2p (*id.,* page 929, citing to Pollack et al., 1993, Gore et al., 1993, Rees-Milton et al., 1997). So, for example, mutations outside of those residues identified as being important for lithium sensitivity are unlikely to affect the lithium binding activity of the protein.

A skilled artisan at the time of the invention would have understood that high conservation of amino acid sequences typically has important functional implications, and therefore highly conserved amino acids within the family should not be mutated if one desires to retain biological function, or alternatively, biological function is more likely to be retained if conservative substitutions are made in these regions. Clearly, a large of amount of structure-function correlation data had been published at the time the application had been filed.

A person skilled in the art would have inferred that, being a member of the family of phosphatases that hydrolyze PAP to AMP, the Hal2p protein is subject to certain well-established rules with respect to catalytic activity. One of skill in the art upon reviewing Albert (and the other references cited in the specification), would have been provided with adequate guidance with respect to making functional mutants of Hal2p containing one conservative amino acid substitution, and that retain a high (90%) level of 3'(2'),5'-bisphosphonate activity.

Extended experimentation is not required to determine which substitutions would be acceptable to retain the 3'(2'),5'- bisphosphate nucleotidase activity claimed in view of the fact that the relationship between the sequence of the Hal2p and its activity are well understood.

Applicants acknowledge that change of a single amino acid can in some instances alter the activity of a protein. However, it is also true that most such changes have no effect whatsoever

on the activity, or at least permit the protein to retain the activity of the referent. Applicants claim only those variants which actually do retain a certain level of 3'(2'),5'-bisphosphonate activity recited in the claims, and thus the claims do not encompass inoperative embodiments. As stated above, assays for these types of biological activity are set forth in the specification in paragraph **[0043]**. Thus, it is very feasible to make a single amino acid change in the protein sequences recited in claim 1 and verify that the variant or fragment retains the desired activity. While it may be unpredictable whether any particular mutant will be successful in retaining activity, it is virtually guaranteed that there will be many instances where such minor changes do result in such retention, and no undue experimentation is required to find them. Accordingly, Applicants believe that the variants recited in the claims are enabled.

In light of the foregoing discussion, Applicants respectfully submit that the specification, combined with the knowledge in the art at the time of the present invention, provides reasonable guidance to the skilled artisan regarding how to make and use the invention, including providing sufficient guidance on protein structure and sufficient guidance on methods for designing variant proteins containing one conservative amino acid substitution and having a desired activity. Accordingly, Applicants respectfully submit that this enablement rejection under 35 U.S.C. § 112, first paragraph may properly be withdrawn.

CONCLUSIONS

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 466992001100. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: June 16, 2010

Respectfully submitted,

By: /Elizabeth Cary Miller/
Elizabeth Cary Miller
Registration No.: 54,708
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 314-7717